Simulation of Cattle Stomach Processes Applied to the Fermentation of Mixed Manure and Straw

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The cattle stomach was considered as the basis for simulating a proposed operation. Microenvironmental degradation mechanisms are understood to be key to the efficient utilization of straw and other resources. Through dynamic tracking of the change law of heat generated by microbial degradation of straw in the cattle stomach, this study used an orthogonal test to explore the optimal ratio of feeding feed, the degradation mechanism in the microenvironment, and the characteristics of cattle manure and straw anaerobic fermentation. The results showed that the number of days of fermentation and the ratio of straw and cattle manure had a significant impact on methane gas production, and the mixture ratio was 1:3, at 26 °C; within 20 days, the cumulative gas production was up to 78.9 L. The results also showed that rumen microorganisms, cattle manure, and mixed straw fermentation can be used at different ratios to obtain the change of methane production, and determine the best ratio to achieve the maximum gas production.

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Keywords: Cattle stomach; Straw degradation; Microenvironment; Cattle manure; Corn straw; Anaerobic fermentation

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INTRODUCTION

A shortage of energy has become a main problem facing global economic development. Straw, as the main source of biomass energy, has the characteristics of rich resources, low price, and environmental favorability, since it can replace non-renewable energy. As a large agricultural and animal husbandry country, China produces many different types of straw annually, and the amount of recycled straw reached more than 1000 million tons, but the straw utilization rate has been relatively low (Dai *et al.* 2021). In addition, China has an increasingly prominent problem of soil pollution that is caused by livestock and poultry manure emissions (Li *et al.* 2020), The potential for these unused biomass energy to be converted into the equivalent of standard coal is one billion tons (Feng *et al.* 2021). Liu *et al.* (2022) established a gas production model of mixed anaerobic fermentation based on a Box-Behnken design. Zhang *et al.* (2016) studied the effect of steel slag micropowder on methane production through anaerobic fermentation of cattle manure. Li *et al.* (2020) studied the feed ratio and semi-continuous anaerobic fermentation of corn straw, and they found that when the ratio of corn straw to cow manure was 1:3, the gas production was at its maximum. Zhang (2011) studied the characteristics of efficient anaerobic fermentation of wheat straw and found that when the ratio of wheat straw to cattle manure was 1:2, the maximum gas production rate was at 40 ℃ and the minimum gas production rate was at 20 °C. Huang (2019) studied the efficient anaerobic fermentation technology of rice straw and found that there was no significant synergistic effect between the pretreated rice straw and sheep or cattle manure. Xu *et al.* (2020) studied the mixing ratio of corn straw and cattle manure separation solution, and they concluded that the maximum yield was achieved under the fermentation test conditions. Jin *et al.* (2021) analyzed the effect of the addition of corn straw on the fermentation of cow manure compost. It was concluded that the ratio of corn straw to cow dung was 2:3 and the seed germination rate could reach 97.35 % after 23 days of fermentation. Cai *et al.* (2022) studied the enhancement of anaerobic fermentation of cattle manure, and the results showed that cellulose pretreatment and micro-voltage have significant influence on anaerobic fermentation of cattle manure. Bułkowska *et al.* (2022) studied the biogas production process with glycerol as the substrate of anaerobic fermentation of cattle manure. Van *et al.* (2022) found that the anaerobic decomposition of wetland grass using cattle manure could reduce carbon dioxide and methane in the biogas. Singh *et al.* (2022) synthesized IONPs (Iron Oxide Nanoparticles, IONPs) using neem leaf extract. Therefore, the effect of methane production by anaerobic fermentation of cattle manure and straw could be improved.

In recent years, many experts and scholars in China and abroad have conducted in-depth research on anaerobic fermentation of cattle manure, but there have been few reports on the application of the gas method. In this paper, bionic simulation and an orthogonal testing were performed for cattle feed matching, and a bionic cattle stomach model was established. The orthogonal test of three factors and three levels was run on the influencing factors of anaerobic fermentation of cattle manure. Further specific analysis showed that at 26 ℃, the corn straw:cattle manure ratio was 1:3. Within 20 days, the maximum cumulative gas production could reach 78.91 L. Thus, the days of fermentation and the ratio of cattle manure to straw had significant effects on methane gas production. The experiment of co-fermentation of cow dung with different types and different ratios of straw and studying the change of methane production can provide a theoretical basis for the construction of a bionic system for efficient and stable control of straw degradation.

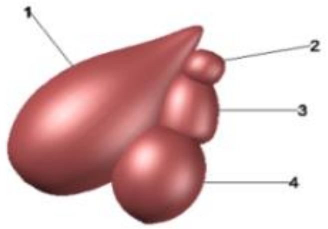
Bionic Digestion System Simulation

The stomach is the place where all animals digest, decompose, and ferment food; thus, a lot of digestion reactions take place. The stomachs of some organisms not only can degrade food, but they produce methane and carbon dioxide. Such a stomach, as in cattle, is called the rumen. It contains a lot of methanogens that produce methane and carbon dioxide through biochemical reactions during food digestion.

The fermentation process of other rumen organisms was explored by simulating the anaerobic fermentation of the cattle stomach. The main parameters of cattle stomach microenvironment include acid-base buffering capacity, volume, *etc.* Basic data of cattle stomach environment is shown in Table 1. A cattle stomach model is shown in Fig. 1.

Table 1. Basic Data of Cattle Stomach Environment (Wang and Mao 2005)

Intragastric Environment of Cattle					
Microambient Temperature (°C)	Acid Base Buffering Capacity	Volume (L)	pH Level	Peristaltic Frequency	Osmotic Pressure (Osm/L)
38 to 41	6.8 to 7.8	About 150 L	6 to 7	1 to 3	260 to 340

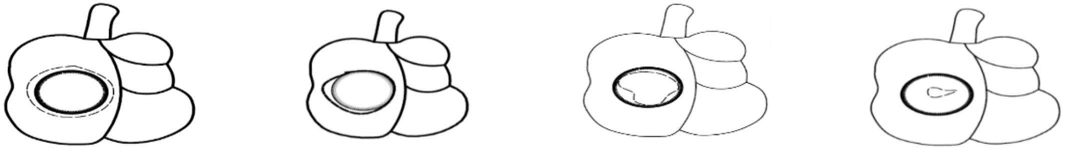


1. Rumen; 2. Reticulum; 3. Omasum; 4. Rennet

Fig. 1. Cattle stomach model

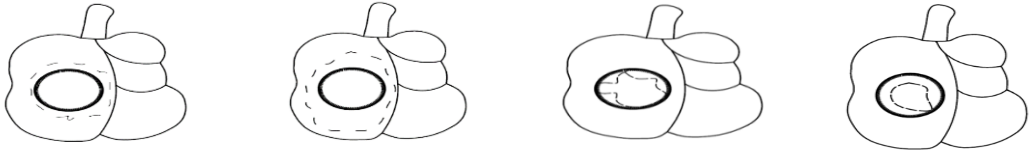
The stomach (Fig. 1) of cattle is the place for digestion of cattle's food intake, consisting of the rumen, reticulum, omasum, and abomasum, in which there are a large number of microorganisms (Pei 2012). These microorganisms can complete many biochemical reactions and maintain various life activities of cattle (Beauchemin *et al.* 2016).

1) Eating green hay



(a) 1 h after eating
(b) 2 h after eating
(c) 3:00 am, contraction
(d) 7:00 am, contraction

2) Eating corn stalk

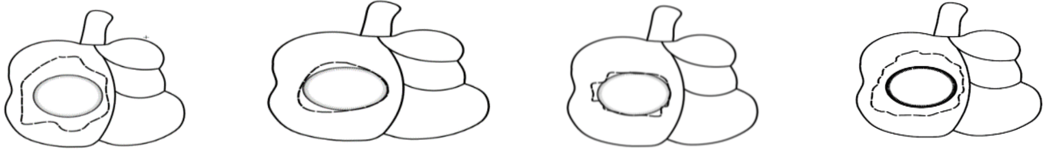


(a) eating 1 h
(b) eating 2.5 h
(c) Digestion completed and contraction
(d) Eating again and contraction

3) Eating wheat straw

(a)1.5 h feeding, expansion
(b) 2 h later, contraction
(c) 2.5 h feeding, expansion
(d) 2 am, contraction

4) Eat rice, sorghum straw



(a) ate rice straw for 3 h
(b) ate rice straw for 0.5 h
(c) ate sorghum straw for 1.5 h
(d) ate sorghum straw for 3 h, and expanded

Fig. 2. Dynamic tracking of feeding changes in cattle stomach

Dynamic Tracking of Microbial Degradation in the Stomach

Based on the principle of bionics, combining biology and biomass energy, it is expected that the application and analysis of rationally fermenting the mixed raw materials of cattle manure and straw to prepare gas production, the cattle were fed with different straw, and the variation of heat generated by microbial degradation of straw in the stomach was dynamically tracked. The differences and similarities of degradation parameters and microenvironment of straw degradation in the cattle stomach were compared and studied.

Mechanism of Anaerobic Fermentation

Cattle stomach microorganisms mainly consist of bacteria, methanogens, fungi, protozoa, and a small number of bacteriophages. The anaerobic fermentation process is shown in Fig. 3.

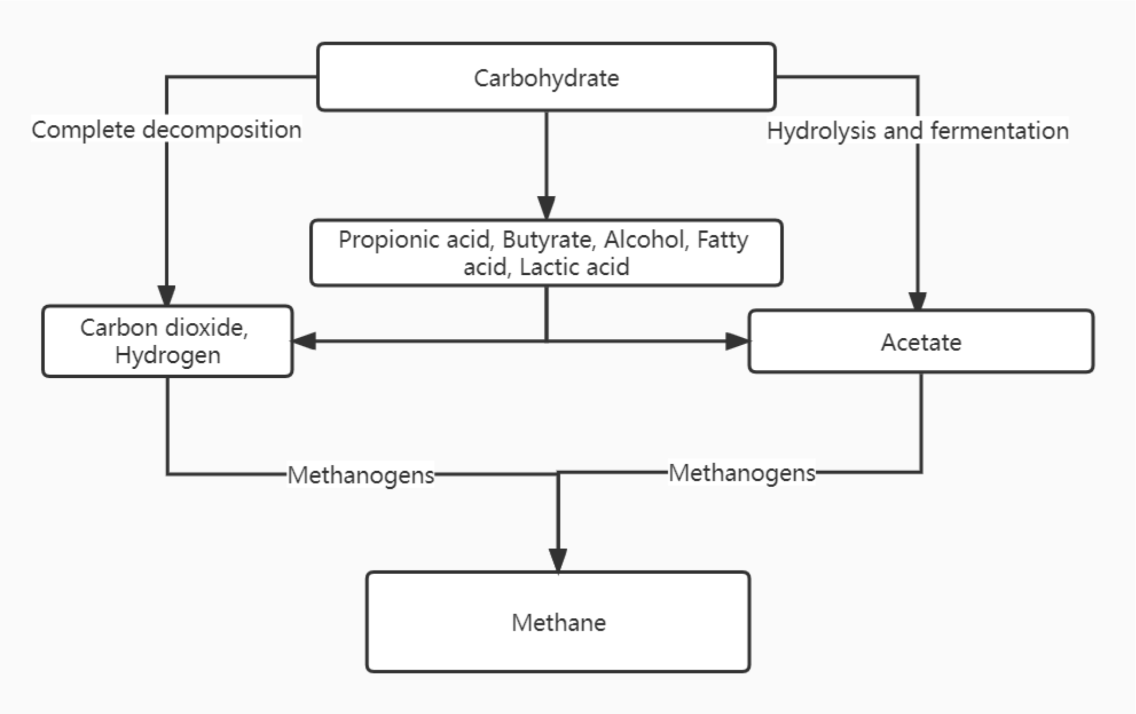


Fig. 3. Anaerobic fermentation process

Production of hydrogen and acetic acid

According to Fig. 3, the reaction equations can be written as follows:

1) Degradation of ethanol:

$$CH_3CH_2OH + H_2O = CH_3COOH + 2H_2 \tag{1}$$

2) Degradation of propionic acid:

$$CH_3CH_2COOH + 2H_2O = CH_3COOH + 3H_2 \tag{2}$$

3) Degradation of butyric acid:

$$CH_3CH_2CH_2COOH + 2H_2O = 2CH_3COOH + 2H_2 \tag{3}$$

Methanogenic stage

According to Fig. 3, the specific reaction equations were as follows (Yang and Chen 2021):

1) Formic acid breaks down to produce methane:

$$HCOO^- + H_2O = CH_4 + HCO_3{}^- \tag{4}$$

2) Acetic acid breaks down to produce methane:

$$CH_3COO^- + H_2O = CH_4 + HCO_3{}^- \tag{5}$$

3) Methanol decomposes to produce methane:

$$4CH_3OH = 3CH_4 + HCO_3{}^- + H^+ + H_2O \tag{6}$$

4) Reduction of carbonate ions with hydrogen:

$$4H_2 + HCO_3{}^- + H^+ = CH_4 + 3H_2O \text{ (main)} \tag{7}$$

Predicted Methane Production

This study employed the findings of earlier experimental work as the basis for simulating and predicting the results of biodegradative processes. Methane production was predicted based on the cellulose, hemicellulose, crude protein, crude fat, and other components. The prediction formula was as follows (Liu and Wang 2022), assuming that the mass of a cattle was 300 kg, the daily intake of food was 15 kg, in which the dry matter was 10 kg, the hemicellulose was 0.5 kg, and the cellulose was 1 kg. The daily gas production forecast was as follows:

$$CH_4 = 18 + 225\varphi\gamma^2 \tag{8}$$

$$CH_4 = -37.5 + 224.7\varphi - 1.9\varphi^2\gamma^2 \tag{9}$$

In these equations, φ is the dry matter intake (kg/d), and γ is a constant (-0.9363);

Through substituting the data into Eqs. 8 and Eq. 9, the daily gas production was predicted to be 385.3 g. Methane gas production is further given as follows:

$$CH_4 = 61.742 + 9.25\theta + 31.5H + 48.01C\gamma^2 \tag{10}$$

$$CH_4 = 0.76 + 1.03F + 0.13N + 0.35P - 0.81E\gamma^2 \tag{11}$$

where H and C are the hemicellulose and cellulose intake, respectively (kg/d). By substituting the data into Eq. 10, the daily gas production was predicted to be 394.7 g. By substituting the data into Eq. 11, the daily gas production was predicted to be 371.1 g.

According to the change of season and temperature, the internal environment of cattle stomach also changes to some extent. Equations 12 and 13 provide the prediction of changes in cattle stomach gas production in spring and winter:

$$CH_4 = 0.3B + 38.11\gamma^2 = 0.95 / CH_4 = 36.31\rho - 3.9\gamma^2 \tag{12}$$

According to Eq. 12, the daily gas production in Spring could reach 392.3 g.

$$CH_4 = 0.15B + 55.81\gamma^2 = 0.61 / CH_4 = 18.11\rho + 53.64\gamma^2 \tag{13}$$

According to Eq. 13, the daily gas production in Winter could reach 387.81 g.

$$CH_4 = 14.56\varphi + 44.92\gamma^2 \tag{14}$$

$$CH_4 = 121.37\varphi + 13.81N\gamma^2 \tag{15}$$

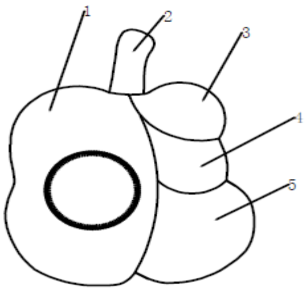
$$CH_4 = 112.88\varphi + 41.37N\gamma^2 \tag{16}$$

$$CH_4 = 43.45\varphi + 0.21N\gamma^2 \tag{17}$$

Equations 14 through 17 were combined so that the daily gas production was 392.7 g. In the above equations, CH_4 is the methane emission (g/d), θ is the soluble intake of neutral detergent (kg/d), F is the crude fiber intake (kg/d), N is the nitrogen free extract intake (kg/d), P is the crude protein intake (kg/d), E is the crude fat intake (kg/d), B is the weight of cattle (kg), and ρ is the digestible dry matter intake (kg/d). According to the calculation, the methane emission of 300 kg cattle was 370 to 395 g per day, indicating that the prediction was in line with the reality.

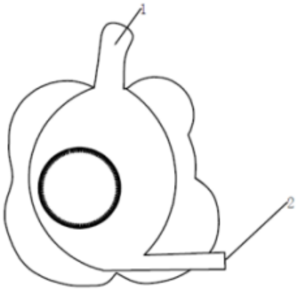
Model building

Rumen is the main site for rumen organisms to conduct anaerobic fermentation. The model of rumen biological anaerobic fermentation was established according to the shape of rumen biological stomach, as shown in Figs. 4 and 5.



1. Rumen 2. Esophagus 3. Reticulum 4. Omasum 5. Rennet

Fig. 4. Schematic diagram of rumen bio-stomach



1. Feed inlet 2. Residue discharge outlet

Fig. 5. Model diagram of rumen bio-stomach

The stomach model of biomimetic rumen animals can be adjusted according to the stomach conditions of different rumen organisms, and the relevant orthogonal test could be conducted.

Simulation and Analysis

In this work, the cattle stomach was simulated and analyzed by ANSYS (ANSYS Company, v.2020, Canonsburg, PA, USA) to study the status of its internal microorganisms. According to the actual shape of the cattle stomach, a 3D model of the front side was drawn. To better highlight the flow state of the cattle stomach, the front side model is shown in Figs. 6 and 7.

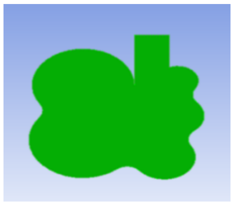


Fig. 6. Physical model of cattle stomach

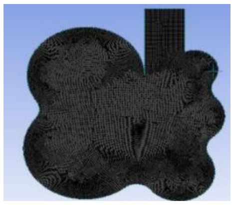


Fig. 7. Mesh model

The simulation started from the feeding of cattle, as shown in Fig. 8. The distribution of temperature in the cattle stomach just after eating is shown in Fig. 9.

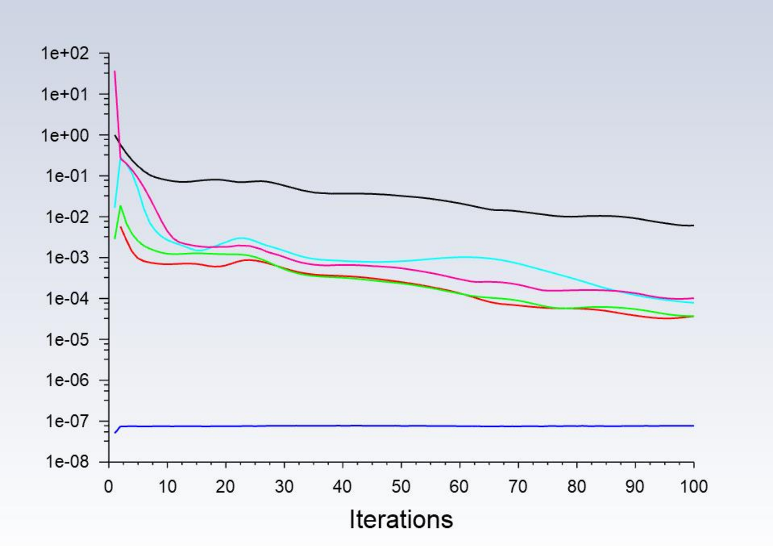


Fig. 8. Changing trend of flow state in the cattle stomach
Note: the black line represents the content flow rate, the pink line represents the feed flow rate, the light blue line represents the microbial flow rate, the green line represents the air flow rate, carbon dioxide flow rate is shown in red, and methane flow rate is shown in blue

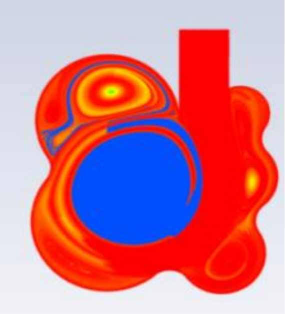


Fig. 9. Distribution of temperature in the cattle stomach just after eating
Note: red means high temperature, blue means low temperature

According to the simulation results of Fig. 8, it can be seen that: at the beginning of feeding, the flow rate of some liquid in the cattle stomach suddenly changed from 0.1 m/s to 20 m/s, and then it dropped to the usual 0.1 m/s after 20 min of feeding. The flow rate of methane was 1×10^{-2} m/s, and then with the digestion of food, the flow rate of methane in the cattle stomach gradually increased. As shown in Fig. 9, just after eating the cattle stomach temperature was 41 °C, and the temperature of the food only 25 °C (ambient temperature).

According to the simulation results: After 10 min of feeding, the gas in the stomach of the cattle had been flowing at a speed of 0.4 m/s. The undigested food was in a solid state with a slow flow rate or even not flowing at all. At the same time, biochemical reactions were also taking place in the digestive juices, which could also produce gases; thus, the situation shown in Fig. 10 occurs.

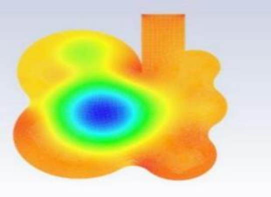


Fig. 10. Change of gas flow rate in cattle stomach after 10 min of feeding
Note: blue means low flow rate, yellow means high flow rate

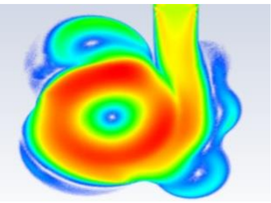


Fig. 11. Change of pressure in cattle stomach after 1 h of feeding
Note: red means high pressure, blue means low pressure

According to the simulation results: one hour after eating, the cattle stomach pressure changes are shown in Fig. 11, the food is present in the center of the cattle stomach, gas diffusion to the cattle stomach around the cattle stomach around the pressure is greater than the cattle stomach center.

SIMULATION EXPERIMENTAL

Microenvironmental Degradation Scheme

Firstly, by analyzing the simulation process, the differences of microbial degradation environment in cattle stomach under different feeding conditions were compared, and the reasons for the differences were analyzed. Then, the differences of microbial degradation in straw microenvironment at different time were analyzed. Finally, the optimal degradation state in the cattle stomach was determined by using the corresponding results of different simulated temperatures, so as to determine the optimal gas production rate.

Optimum Feed Ratio

Simulation methods: 1) The cattle of the same type, age, and weight were simulated, and different kinds of feed were simulated for feeding. According to the changes of gas content parameters in the simulated cattle stomach, the optimal feed ratio was determined. 2) The best feed ratio was put into the bionic stomach for simulated culture.

Table 2. Microenvironment of Cattle Stomach under Different Conditions

		CH_4 (mL)	Peristaltic Frequency	pH Level	Degree of Microbial Activity
1 h	Wheat Straw	3415.68	2	6.4	Inactive
	Maize Straw	3675.23	2	6.2	Very Active
	Rice Straw	3534.64	2	6.0	Active
1.5 h	Wheat Straw	6979.52	2	6.0	Active
	Maize Straw	7369.65	3	6.3	Very Active
	Rice Straw	6591.45	2	6.1	Active
2 h	Wheat Straw	5067.42	2	6.5	Inactive
	Maize Straw	5343.15	3	6.1	Active
	Rice Straw	5103.97	2	5.8	Inactive

Simulation steps: The simulation used the same proportion of feed to feed cattle of the same type, age, and weight, and observed and recorded changes in parameters such as daily methane production, peristaltic frequency, pH, and microbial activity during the simulation (results are shown in Table 2) (Li 2018).

According to the comparison of simulation experiments and summary findings, the fermentation of corn straw in the cattle stomach was the most active microorganism at about 1.5 h. Because of the loose and porous structure inside corn straw, it could contact better with cattle stomach contents, and thus produce a better reaction effect. The decrease of gas production after 2 h was caused by the depletion of feed and the decrease of bacterial activity.

According to previous experts and scholars (Wang *et al.* 2016; Zhou *et al.* 2019), with the formula (feed formula as shown in the table below) for analysis and contrast.

Table 3. Formula 1 of Cattle Feed

	A1	B1	B2	C1	C2	C3	D1	E1
Formula 1	70	9	8	9	1.6	0.8	0.6	100
Formula 2	75	9	8	5	1.6	0.8	0.6	100
Formula 3	80	9	3	5	1.6	0.8	0.6	100
Formula 4	85	4	3	5	1.6	0.8	0.6	100
Formula 5	90	2	1	4	1.6	0.8	0.6	100

A1, corn stalk; B1, bran; B2, bean cake ; C1, corn meal; C2, bone meal; C3, shell meal; D1, salt ; E1, total, all values in the table are %.

Table 4. Formula 2 of Cattle Feed **(**Wang *et al.* 2016)

	A1 (kg)	B1 (kg)	B2 (kg)	C1 (kg)	C2 (g)	C3 (g)
Formula 1	30	0.5	0.5	0.5	60	40
Formula 2	35	0.33	0.33	0.35	60	40
Formula 3	40	0.5	0.5	0.5	75	50
Formula 4	40	0.5	1	0.5	75	50
Formula 5	11	0.75	0.75	0.87	75	50
Formula 6	11	0.75	0.75	0.87	45	35

Notes: A1, green hay; B1, bran; B2, bean cake; C1, corn meal; C2, bone meal; C3, salt

Table 5. Formula 3 of Cattle Feed (Zhou *et al.* 2019)

	A1	A2	B1	B2	B3	C1	C2	C3	D1
Formula 1	40.81	——	24.48	5.50	11.26	0.01	0.02	17.91	0.01
Formula 2	——	19.13	33.58	0.65	7.07	0.01	0.02	39.53	0.01
Formula 3	12.11	12.57	31.99	1.14	8.16	0.01	0.02	33.99	0.01
Formula 4	38.00	——	36.00	5.70	6.14	1.16	0.50	12.00	0.50
Formula 5	——	34.00	36.00	7.20	4.64	1.16	0.50	16.00	0.50
Formula 6	14.00	21.00	36.00	6.00	5.84	1.16	0.50	15.00	0.50
Formula 7	36.00	——	46.00	6.00	5.75	1.25	0.50	4.00	0.50
Formula 8	——	30.00	46.00	6.00	3.74	1.26	0.50	10.00	0.50
Formula 9	12.00	20.00	46.00	6.00	5.76	1.24	0.50	8.00	0.50

Notes: A1, maize straw; A2, wheat straw ; B1, corn ; B2, bran; C1 powder; C2 salt; C3, alfalfa; D1, premix, all values in the table are %

The simulation analysis showed that the Formula 2 in Table 3 contributed most to the growth and development of cattle, and Formula 3 in Table 3 promoted more CH_4 in the stomach. In Table 3, Formula 5 fed the cattle in the stomach when the microbial was most active. Formula 4 in Table 4 could maintain the most stable pH value in the cattle stomach, which was 6.3. Formula 1 in Table 4 could keep peristalsis frequency of cattle stomach.

Orthogonal Test Verification

Simulation test method

The conditions in the bionic cow stomach were simulated by ANSYS, and the composition, pH and temperature of different straws were simulated. No other conditions were changed during the simulation process, so as to generate gas generation data during the simulation process.

Simulation test design

After simulating the conditions of the bionic cattle stomach, the method was applied to simulate 15 kg of different kinds of crushed straw, temperature, and pH conditions. The change of gas production during simulation was observed after 2.5 h to determine the best fermentation scheme.

Simulation test results

With straw type, temperature, and pH as the observation objects, three levels were set for each object, and nine experiments were conducted to observe the parameters changes in the cattle stomach by simulation the crushed straw, pH, and temperature of different kinds in the stomach at the same time. The influencing factors of the orthogonal test are shown in Table 6, and the test results are shown in Table 7.

Table 6. Influencing Factors and Levels in the Test

Level	Factor		
	A, Types of Straw	B, Temperature (°C)	C, pH
1	Corn straw	38	6.4
2	Wheat straw	39	6.6
3	Rice straw	40	6.8

Table 7. Test Results

Testing Order Number	A, Types of Straw	B, Temperature (°C)	C, pH	Gas Production (L)
1	1	1	3	90
2	1	2	2	93.2
3	1	3	1	98.7
4	2	1	2	83.3
5	2	2	1	79.6
6	2	3	3	89.2
7	3	1	1	73.4
8	3	2	3	85.3
9	3	3	2	79.4
K1	281.9	246.7	251.7	
K2	252.1	258.1	255.9	
K3	238.1	267.3	264.5	
k1	83.97	82.2	83.9	
k2	84.0	86.0	85.3	
k3	79.4	89.1	88.2	
R	4.6	6.9	4.3	

K_i(i =1,2,3) represents the actual gas production of a factor at the level of i, K_i represents the average value of a factor at the level of i, $R = K_i(\max) - k_i\ (\min)$. A larger R value means the influence of this factor on gas production was more obvious.

Table 8. Significance and Variance

Source	Quadratic Sum	Degrees of Freedom	Mean Square Error	F	Significance
A	333.61	2	166.81	5.85	Significance
B	70.996	2	35.50	1.25	No Significance
C	28.382	2	14.19	0.50	No Significance

Note: significant < 0.05 significant, significant > 0.05 not significant

Analysis of Simulation Test Results and Influencing Factors

The results showed that when the temperature in the cattle stomach was 40 ℃, pH 6.4, and the digested food was corn stalk, the gas production was predicted to be the highest. It could be seen that straw type had the greatest influence on methane yield, followed by temperature and pH. Corn straw was easy to promote methane production because it was loose and porous, and could come into better contact with the contents of cattle stomach, so that the reaction was more complete and thorough. Both wheat straw and rice straw were empty shells that were not easy to be digested in cattle stomach, so the fermentation gas production was less than corn straw.

Although there was a certain error (3.72%), the error was less than 5%. The data of the orthogonal test was more accurate and detailed. The orthogonal test was the verification of the simulation experiment, where an orthogonal experiment provides more data support for simulation experiment, the two complement each other.

Testing of the Bionic Stomach Model

In order to verify whether the bionic cattle stomach can work properly, the environment and feed of the bionic cattle stomach was first simulated (Huang *et al*. 2021; Shi *et al.* 2022), the digestion time during the simulation was observed, the digestion conditions were detected, and the design of the bionic cattle stomach was understood. Whether reasonable, and in the simulated digestion process to add different percentages of digestive enzyme characteristics, observe the change of digestion time.

Table 9. Relationship between Enzyme Ratio and Digestion Time

	No Enzymes	5% Enzymes	10% Enzymes	15% Enzymes	20% Enzymes
Time	2.5 h	2.3 h	1.9 h	1.5 h	1.5 h

Simulation results: Complete decomposition of straw feed after addition of 15% enzyme took 1.5 h, and complete decomposition of straw feed without enzyme took 2.5 h

Table 9 shows that the digestion time of the simulated addition of 15% enzyme was the same as that of the simulated addition of 20% and 25% enzymes. In order to maximize the methane production efficiency of the cattle stomach model, 15% enzyme catalyst can be artificially added during the actual digestion operation.

Cattle Manure and Mixed Straws Raw Material Fermentation to Produce Gas Optimal Anaerobic Fermentation Conditions

In order to determine the straw with the highest gas production, a control method was simulated to co-ferment cow manure and straw in different proportions, and the changes in gas production were observed at all times (Li *et al*. 2020).

According to the results in Table 10, when the ratio of corn straw to cattle manure was 1:3, the gas production of anaerobic fermentation was the maximum on the 15th day, which was 3.91 L. The gas production of wheat straw and rice straw was low. Because of the loose and porous structure inside corn straw, it could better contact with cattle manure, so that the reaction was more sufficient, but the proportion should be moderate. Wheat straw and rice straw were hollow inside, it was not in good contact with cattle manure, so that their gas generation capacity was weaker than corn straw (Liu *et al*. 2017).

The amount of methane produced by fermentation of different straw and cattle manure is shown in Table 10.

Table 10. Amount of Methane Produced by Fermentation of Different Straw and Cattle Manure (25 °C)

	Straw Stalk Proportion	3rd Day (L/d)	6th Day (L/d)	9th Day (L/d)	12th Day (L/d)	15th Day (L/d)	18th Day (L/d)
Cattle manure + Rice straw mixture	1:2	2.32	2.93	3.04	3.02	3.06	3.05
	1:2.5	2.41	3.01	3.21	3.24	3.15	3.17
	1:3	2.23	2.98	3.01	3.04	3.02	3.05
	1:3.5	2.17	2.64	2.71	2.73	2.72	2.73
	1:4	2.20	2.58	2.63	2.61	2.65	2.60
Cattle manure + Wheat straw mixture	1:2	2.25	2.87	2.90	2.85	2.86	2.88
	1:2.5	2.37	3.01	3.05	3.08	3.10	3.07
	1:3	2.45	3.07	3.10	3.11	3.09	3.10
	1:3.5	2.51	3.12	3.17	3.16	3.14	3.16
	1:4	2.42	3.05	3.03	3.02	3.06	3.08
Cattle manure + Corn straw mixture	1:2	2.13	2.76	3.25	3.48	3.67	3.54
	1:2.5	2.24	2.81	3.36	3.54	3.78	3.73
	1:3	2.65	3.12	3.43	3.71	3.91	3.82
	1:3.5	2.40	2.91	3.51	3.63	3.85	3.64
	1:4	2.31	2.88	3.36	3.58	3.79	3.71

Determine the Best Fermentation Temperature

In order to determine the optimal temperature of co-fermentation, the same amount of cow dung and straw were co-fermented under different constant temperature conditions by ANSYS simulating method, and the change of gas production was observed at all times.

The data obtained are shown in Table 11. Changes in gas production during maize straw and cattle manure fermentation with different ratios at 26 °C is shown in Fig. 12.

Table 11. Relationship between Temperature and Methane Production

	Gas production at different temperatures (L)					
	23 °C	24 °C	25 °C	26 °C	27 °C	28 °C
10th day	3.79	3.80	3.84	3.86	3.83	3.82
15th day	3.88	3.91	3.92	3.95	3.92	3.91
20th day	3.90	3.92	3.95	3.97	3.94	3.93

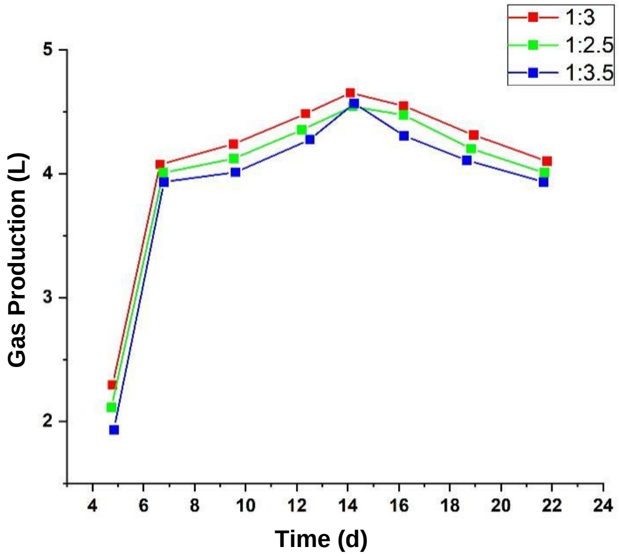


Fig. 12. Changes in gas production during maize straw and cattle manure fermentation with different ratios at 26 °C

According to Fig. 12, within the first nine days, the gas production was not stable and steadily improved. From the 13th day, the gas production gradually tended to be stable, and on the 15th day, the daily gas production reached the maximum, and then it slightly decreased. In general, the daily gas output is the highest at 26 ℃.

Orthogonal Test- Test Method

During the design test, simulated cattle manure and straw were fully mixed in different proportions, and orthogonal experiments with three factors and three levels were carried out on this basis, which was, simulated fermentation was completed in a closed container, and simulated the gas production during fermentation was compared with the temperature and fermentation time.

Test materials

The physical and chemical parameters of cattle manure and corn straw simulated in the orthogonal test refer to the reserve straw (0.4 to 0.7cm) of the suburban farm in Luoyang, China and the cattle manure (4 °C incubator, pre-prepared bionic food residues after digestion in the cattle stomach) was taken from the above bionic cattle stomach, The physical and chemical properties of simulated cattle manure and corn straw are shown in Table 12.

Table 12. Physical and Chemical Properties of Corn Straw and Cattle Manure

Ingredient	Organic Carbon (%)	TN (%)	C/N	Moisture Content (%)
Corn Straw	44.91	0.96	47.72	11.23
Cattle Manure	42.45	1.87	21.31	70.45

Note: TN (total nitrogen) represents the total nitrogen, and C/N (Carbon /nitrogen) represents the ratio of carbon to nitrogen

In the fermentation process of cattle manure and straw, microorganisms will be more active and consume a large amount of nutrients such as organic matter and nitrogen. Therefore, the larger the C/N, TN, and Organic Carbon, the better the fermentation effect. Moisture content can provide a more suitable microenvironment for microorganisms, so water content is closely related to cattle manure fermentation.

Test Design and Results

Temperature (A), time (B), and the ratio of straw: Cattle manure (C) were set as the independent variables, and three levels were set for each factor. The volume of cattle manure and corn straw was 30 m^3 in a preset proportion, and then fermentation was completed in the same device. Nine experiments started and ended at the same time. The parameter k_i (i = 1, 2, 3) represents the actual gas production of a factor at the level of i, k_i represents the average value of a factor at the level of i, $R = k_i(\max) - k_i(\min)$ (Wu *et al.* 2015; Dou *et al.* 2018), the greater R value meant the influence of this factor on gas production was more obvious. The influencing factors' levels and results of orthogonal test are shown in Tables 13 and 14, respectively. The predicted gas production value could be predicted by Design-Expert (Stat-Ease, Design-Expert 12, Minneapolis, MN, USA) response analysis test software to obtain variance analysis as shown in Table 15. Meanwhile, the variation of anaerobic fermentation gas production caused by different days and time was analyzed as shown in Figs. 13 and 14.

Table 13. Influencing Factors and Levels of Orthogonal Test

Level	Factor		
	A Temperature (T, °C)	B Time (t,d)	C Straw: Cattle Manure
1	24	10	1:2.5
2	25	15	1:3.0
3	26	20	1:3.5

Table 14. Orthogonal Test Results

Testing Order Number	A Temperature (T, °C)	B Time (t, d)	C Straw: Cattle Manure	Actual Gas Production (L)	Estimated Gas Production (L)	Residual Error
1	1	1	3	37.73	38.12	-0.39
2	1	2	2	59.32	58.65	0.67
3	1	3	1	70.30	78.40	-8.1
4	2	1	2	38.54	38.46	0.08
5	2	3	1	58.31	58.80	-0.49
6	2	2	3	72.61	79.43	-6.82
7	3	2	1	38.73	38.60	0.13
8	3	1	3	58.96	59.25	-0.29
9	3	3	2	79.67	79.42	-0.25
K1	173.35	176.36	176.46			
K2	115	234.38	176.59			
K3	176.3	173.34	176.53			
k1	58.39	59.09	58.82			
k2	38.39	79.08	58.86			
k3	58.93	58.6	58.84			
R	20.7	20.33	0.04			

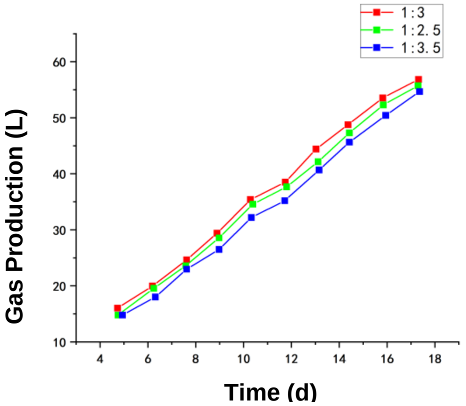


Fig. 13. Change of cumulative gas production by anaerobic fermentation (per d)

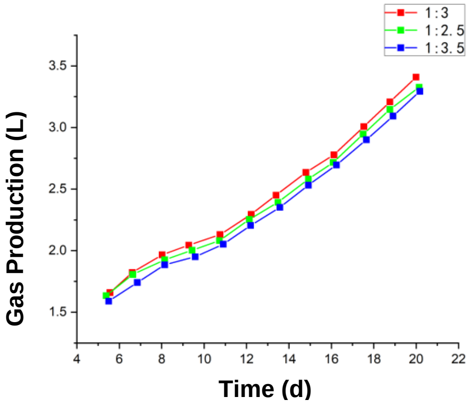


Fig. 14. Change of cumulative gas production by anaerobic fermentation (per h)

According to range analysis in Tables 13 and 14, time had the greatest influence on gas production, followed by temperature, and the ratio of straw to cattle manure had the least influence. Since range analysis could not reflect the fluctuation of data, data in Table 14 needed to be analyzed by SPSS STATISTICS (IBM Corp., Armonk, NY, USA) curve estimation and regression analysis, and Table 15 reflects the results of variance analysis.

According to Figs. 13 and 14, the ratio of straw to cattle manure was different, but the gas production trend of anaerobic fermentation did not change, indicating that the ratio of cattle manure to straw was different, and the gas production was different because of different promotion degree, which did not change the reaction in the fermentation process.

Analysis of test results and influencing factors

According to variance analysis, fermentation days had the greatest influence on gas production. In the process of contact between cattle manure and straw, methanogens, yeasts, and lactic acid bacteria could be promoted to reproduce, which played a valuable role in the fermentation of straw and cattle manure. Nonetheless, as time goes by, the impurities produced by cattle manure and straw in the fermentation process increased, leading to a gradual decrease in gas production. The methane content produced by anaerobic fermentation was not obvious by temperature and manure to straw ratio (Hu *et al.* 2022; Pu *et al.* 2022).

Table 15. Analysis of Variance

Objective	Source	Quadratic Sum	Degrees of Freedom	Mean Square Error	F	Significance
Gas Production	A	80.458	2	40.229	0.15	No significance
	B	475.481	2	237.740	1.259	Significance
	C	77.897	2	38.949	0.145	No significance

Significant < 0.05 significance, significant > 0.05 no significance

According to variance analysis, time had a significant effect on gas production, but the effect of temperature and manure to straw ratio on gas production was relatively insignificant. It could be seen from Table 14 that gas production reached the optimal state when the temperature was 26 °C and the ratio of corn straw to cattle manure was 1:3, but the result at this time could not be considered as absolute optimal.

Due to the loose and porous structure inside the corn straw, it can better contact with cow manure, but there is an optimal ratio. If there is too much cow manure at other times, the corn straw is too few and cannot be better fermented; conversely, when there is too much corn straw, it can not be better contact with cow manure. Therefore, the ratio of corn straw to cattle manure was 1:3 is more appropriate.

Establish Regression Model

To better study the order and significance of the effects in the orthogonal test and better predict the results of the test, multiple linear regression analysis (Guo *et al.* 2010) was specially conducted on the test results to establish the regression equation:

For the index temperature T_i for the regression equation was established:

$$T_i = 65.59 + 10.35A \tag{18}$$

For the index time t_i, the regression equation was established:

$$t_i = 65.59 + 10.165B \tag{19}$$

For the index corn straw to cattle manure W_i, the regression equation was established:

$$W_i = 58.84 + 0.02C \tag{20}$$

Through comparison of the regression coefficients of each curve, it could be observed that the influence of each factor was essentially the same as that of the range influencing factor, with F value and significance basically the same. The R^2 value of the regression factor was only the ratio of cattle manure:corn straw, which was less than 0.9, and the accurate fitting degree was intermediate.

ANALYSIS OF ORTHOGONAL TEST RESULTS

The following factors affecting the fermentation of cattle manure were analyzed: fermentation days, fermentation time, and the ratio of cattle manure to straw. Then, the predictive regression equation of the correlation between the three factors and the gas production of cattle manure fermentation was established.

The three-factor and three-level orthogonal test was conducted on the fermentation of cattle manure. The results of orthogonal test and variance analysis showed that the methane yield could reach 3.91 L per day when the temperature of cattle manure and straw was 26 °C, the fermentation time was 20 days, and the ratio of corn straw to cattle manure was 1:3. Under these conditions, gas production was considered to reach the maximum.

The ratio of straw to cow dung only changed the amount of daily gas production, but it did not change the general trend of gas production, indicating that the strength of the synergistic fermentation of cow dung and corn straw was related to the ratio of manure to straw.

CONCLUSIONS

1. Through the comparative analysis of multiple groups of data, the optimal ratio of cattle stomach feed to digestion and methane gas generation in cattle stomach was obtained: corn 80%, bran 9%, bean cake 3%, corn meal 5%, bone meal 1.6%, shell meal 0.8%, and salt 0.6%.
2. Fluent simulation was used to simulate the flow state of the cattle stomach, as well as the change in the temperature and pressure of the cattle stomach after eating to obtain the curves of the flow situation of the cattle stomach at different stages and the change diagram.
3. The orthogonal testing of three factors and three levels was used to explore the systematic fermentation effect of cattle manure and straw. Through analysis of variance and significance, it was concluded that gas production could reach the maximum under the condition of temperature of 26 ℃, fermentation time of 20 days, and corn straw to cattle manure ratio of 1:3.

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